

# Measurements of the Octanol/Water Partition Coefficient by Chromatographic Methods

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A theoretical relationship is developed to provide a quantitative definition of hydrophobicity using established theoretical and semi-empirical relationships. A method of predicting partition coefficients of relatively water-insoluble third components between water and an immiscible second component is devised and tested. Comparison with experimental data for four classes of compounds in the water/*n*-octanol system at 25° C shows excellent agreement, indicating that values for substances for which direct determination is experimentally precluded can be calculated with confidence.

**Key words:** Activity coefficients; alkybenzenes; gas chromatography; octanol/water partition coefficients.

## 1. Introduction

In recent years there has been an increased interest in the use of hydrophobic parameters to study the fate of toxic substances in the marine environment since the ability of organic compounds to bioconcentrate is believed to depend upon the partition behaviour of molecules between lipid and aqueous phases [1,2].<sup>1</sup> An important and simplifying observation has been that by Neely, Branson and Blau [1] who demonstrated that bioconcentration factors for chlorobenzenes and chlorophenols between trout muscles and dilute solutions in water could be successfully correlated with their partition coefficients in the *n*-octanol/water system,  $K_{o/w}$ . Subsequently, Dunn and Hansch [3] compiled hydrophobic interaction data for a large number of organic compounds and showed that these could, indeed, be quantitatively correlated with partition coefficients of organic/water systems. The weight of evidence has led Leo [4] to suggest not only that hydrophobicity is the most important parameter in bioaccumulation and biotransport but that this can be confidently determined in terms of octanol/water partition coefficients.

Because of the thousands of compounds being studied as potential hazards to the environment through bioac-

cumulation, simple economics makes it desirable to devise some system whereby we measure values of  $K_{o/w}$  for key compounds which may be used to calculate values for related compounds. In the only approach to date, Hansch, Quinlan and Lawrence [5] have developed a method for estimating  $K_{o/w}$  based on additive group contributions; these group contributions or "Π values" being as defined by eq (1),

$$\Pi_x = \log K_{o/w}^x - \log K_{o/w}^h \quad (1)$$

where  $K_{o/w}^x$  and  $K_{o/w}^h$  are the octanol/water partition coefficients for the derivative and the parent compound, respectively. Not surprisingly, in the light of the numerous such correlations established in GLC studies [6], Π values are often additive, and the method has met with acceptance. However, because of steric, electronic and hydrogen bonding effects there are many series of compounds for which the method fails. Some alternative would, therefore, be useful, particularly if the correlation method involved real and measurable physical properties of the molecules concerned rather than a purely empirical set of parameters. This paper outlines one such approach and an indication of its applicability.

Partition coefficients are generally determined by some variant on the traditional shake-flask method. This method is slow, tedious, often wasteful, and demanding in the standard of purity of materials it requires. Conse-

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<sup>1</sup>Figures in brackets indicated literature references at the end of this paper.

quently there have been many attempts to develop chromatographic methods to which, in principle, and normally in practice, none of the above objections apply. In the method presented in this paper  $K_{o/w}$  is defined, as proved later, by eq (2),

$$K_{o/w} = \gamma_{\phi}^w / \gamma_{\phi}^o \quad (2)$$

where  $\gamma_{\phi}^w$  and  $\gamma_{\phi}^o$  are the activity coefficients at infinite dilution, based on volume fraction, for the solute in water and *n*-octanol, respectively. The quantity,  $\gamma_{\phi}^w$ , is calculated from the solute aqueous solubility,  $C_w$ , and the solute molar volume,  $V[7]$ , and  $\gamma_{\phi}^o$  is determined from the corrected retention volume of the solute eluting from a column containing *n*-octanol as the stationary phase. Thus  $K_{o/w}$  (at infinite dilution) can be calculated from  $\gamma_{\phi}^w$  and  $\gamma_{\phi}^o$ , which can be measured by two independent methods having all the advantages of the chromatographic approach, while the recognition of eq (2) opens the route to the alternative approach to be described.

## 2. Theoretical

The octanol/water partition coefficient,  $K_{o/w}$ , is defined as the equilibrium ratio of the molar concentration of solute *x* in octanol,  $C_o$ , and the concentration in water,  $C_w$ , in an octanol/water system, viz.

$$K_{o/w} = \frac{C_o}{C_w} \quad (3)$$

But, self evidently,  $K_{o/w}$  may also be defined by eq (4)

$$K_{o/w} = \frac{K_{a/o}}{K_{a/w}} \quad (4)$$

where  $K_{a/o}$  is the air/octanol (saturated with water) and  $K_{a/w}$  is the air/water (saturated with octanol) partition coefficient. GLC theory yields the expression for  $K_{a/o}$

$$K_{a/o} = \frac{C_o RT}{P} = \frac{n_x RT}{(V_x + V_o + V_w) P} \quad (5)$$

where  $n_x$  is the number of moles of solute *x* in the octanol (saturated with water),  $V_x$ ,  $V_o$  and  $V_w$  are the volumes of solute, octanol and water, in the octanol phase, respectively,  $R$  is the gas constant, and  $P$  is the partial pressure of solute *x* above the solution at temperature,  $T$ .

For an ideal vapour the ratio  $P/P^\circ$ , where  $P^\circ$  is the solute saturation vapour pressure, is equal to the activity (*a*). We may define the activity in terms of any quantity that defines relative amount, and an appropriate activity

coefficient, e.g., we may write,

$$a = \gamma_x x = \gamma_{\phi} \phi = \gamma_w W = \gamma_c C / C^\circ \quad (6)$$

where *x* represents mole fraction,  $\phi$  the volume fraction,  $W$  the weight fraction, and  $C/C^\circ$  the ratio of concentration in solution to that in the pure solute liquid. Although the first of these definitions is the one most widely used we choose the second for reasons that will emerge.

Thus, setting

$$\frac{P}{P^\circ} = \phi \gamma_{\phi} \quad (7)$$

substitution for  $P$  in eq (5) yields

$$K_{a/o} = \frac{n_w}{V_x + V_o + V_w} \cdot \frac{RI}{\gamma_{\phi}^o \phi P^\circ} = \frac{RT}{V(x) \gamma_{\phi}^o P^\circ} \quad (8)$$

where  $\gamma_{\phi}^w$  is the activity coefficient in water saturated with *n*-octanol. Provided there is no significant excess volume of mixing in either solvent, the normal solution,

$$K_{a/w} = \frac{RT}{V(x) \gamma_{\phi}^w P^\circ} \quad (9)$$

where  $\gamma_{\phi}^w$  is the activity coefficient in water saturated with *n*-octanol. Provided there is no significant excess volume of mixing in either solvent, the normal solution,

$$K_{o/w} = \gamma_{\phi}^w / \gamma_{\phi}^o \quad (10)$$

It remains only to emphasize again that  $\gamma_{\phi}^o$  and  $\gamma_{\phi}^w$  are values. An immediate and obvious attraction of eq (10) is the absence of explicit solvent parameters, which is not the case if  $\gamma_x$  is used.

## 3. Dependence of the thermodynamic functions associated with solute partitioning between an organic and an aqueous phase on vapour pressure.

Hoare and Purnell [8] have shown that for solutes of similar chemical structure the GLC specific retention volume of solute *x*,  $V_g(x)$ , is related to the saturation vapour pressure of the solute,  $P^\circ$ , by the expression

$$\log V_g(x) = -a \log P^\circ + \text{constant} \quad (11)$$

where *a* is a series constant. The validity of eq (11) has subsequently been further established for a wide range of

chemical types of both solvent and solute [9, 10, 11] and in a summary by Purnell [8]. In so far as data are available there seems to be no recorded exception to the rule. Further since

$$V_g^o(x) = \frac{RT}{\gamma_\phi^o P^o V(x)q}$$

where  $q$  is the solvent density, eq (11) yields

$$\log \gamma_\phi^o V(x) = (a^o - 1) \log P^o + \text{constant} \quad (12)$$

where  $a^o$  is the slope of the  $\log V_g^o(x)$  vs  $\log P^o$  plot for each series of solutes in  $n$ -octanol.

In similar manner we obtain

$$\log \gamma_\phi^w V(x) = (a^w - 1) \log P^o + \text{constant} \quad (13)$$

where  $a^w$  is the slope of the  $\log V_g(x)$  vs  $\log P^o$  plot for each series of solutes in water. Combining eqs (10), (12), and (13) we obtain

$$\log K_{o/w} = (a^o - a^w) \log P^o + \text{constant} \quad (14)$$

Thus for solutes of similar chemical structure the thermodynamic functions,  $\log P^o$ ,  $\log \gamma_\phi^o V(x)$  and  $\log \gamma_\phi^w V(x)$ , associated with the partitioning of a solute between an organic and aqueous phase may each be expressed in the form

$$\text{function} = B + A \log P \quad (15)$$

where  $B$  and  $A$  are numerically defined by the function being considered.

## 4. Results

The solute activity coefficients in octanol,  $\gamma_\phi^o$  and in water  $\gamma_\phi^w$  listed in columns 1 and 2 of table 1 were obtained from an earlier publication of Wasik *et al* (12).  $\gamma_\phi^o$  were calculated from the solute specific retention volume  $V_g^o$ , obtained from retention times of solutes eluting from a GC column containing  $n$ -octanol as the stationary phase.  $\gamma_\phi^w$  values were calculated from solubility data using the following equation

$$\gamma_\phi^w = \frac{1}{\phi_w} \quad (16)$$

where  $\phi_w$  is the solute aqueous solubility in volume fraction. The last two columns show a comparison between the calculated  $K_{o/w}$  (using eq (10)) and the experimental values. The agreement between the two sets of data is excellent.

TABLE 1. Solute Activity Coefficients and Octanol/Water Partition Coefficients at 25.0°C.

Solute	Log $\gamma_\phi^o$ (GLC)	Log $\gamma_\phi^w$ (Generator column /HPLC or GC)	Log $K_{o/w}$	
			Calculated <sup>a</sup>	Experimental
n-Pentane	0.555	4.19	3.63 (3.68)	3.62
n-Hexane	.530	4.73	4.20 (4.22)	4.11
n-Heptane	.517	5.28	4.76 (4.77)	4.66
n-Octane	.512	5.80	5.29 (5.29)	5.18
1-Hexene	.504	3.98	3.48 (3.47)	3.39
1-Heptene	.491	4.58	4.09 (3.07)	3.99
1-Octene	.479	5.24	4.76 (4.73)	4.57
1-Nonene	.470	5.81	5.34 (5.30)	5.15
Toluene	.509	3.17	2.66 (2.66)	2.65
Ethylbenzene	.505	3.66	3.15 (3.15)	3.13
n-Propylbenzene	.494	4.22	3.73 (3.71)	3.69
Ethylacetate	.621	1.15	0.53 (0.64)	0.68
n-Propylacetate	.534	1.64	1.11 (1.13)	1.24
n-Butylacetate	.425	2.15	1.73 (1.64)	1.82

<sup>a</sup>Values in parentheses were calculated using the hydrophobicity equation (eq 17).

## 5. Discussion

Our approach of defining  $K_{o/w}$  in terms of volume fraction based activity coefficients has several important consequences. First, the infinite dilution coefficients  $\log K_{o/w}$ ,  $\log \gamma_\phi^o$ , and  $\log \gamma_\phi^w$  for a given solute type are clearly described by a linear relationship with the solute saturation vapour pressure.

The extent to which this is true may be gauged by consideration of the values of the correlation coefficients,  $r$ , listed in table 2 derived from data of table 1 via linear regression as a consequence of the above.

The data in table 1 indicate that values of  $\log \gamma_\phi^w$  are large and change rapidly with  $\log P^o$ ; whereas,  $\log \gamma_\phi^o$  are much smaller and remain fairly constant ( $0.510 \pm 0.045$ ) for all compounds. Thus the relevant values of  $\log K_{o/w}$  are determined by  $\log \gamma_\phi^w$ . Our approach leads us to a quantitative theoretical definition of the hitherto empirical concept of hydrophobicity,  $H$ ,

$$H = \log K_{o/w} = \log \gamma_\phi^w - 0.510 \quad (17)$$

$$= \log \gamma_\phi^w - k$$

The numerical constant  $k$  will vary for different solvents which means that  $H$  can be defined with respect to solvents other than  $n$ -octanol. The values of hydrophobicity ( $H$ ) calculated using eq (17) are listed in table 1 in parenthesis. A quantitative definition of  $H$  is

TABLE 2. Coefficients of the Regression Equation and the Coefficient of Correlation for the Solute

Type of Correlation	Type of Solute	Slope	Intercept	Coefficient of Correlation (r)
$\log K_{o/w} = A \log P^\circ + B$	Alkanes	-0.9957	6.298	0.999
	Alkenes	-1.1400	5.981	.999
	Aromatics	-0.8809	3.770	.998
	Acetates	-0.7700	2.484	.999
$\log K_{o/w} = k_1 n_c + k_2$	Alkanes	0.5230	0.993	0.999
	Alkenes	.5860	-0.1200	.999
	Aromatics	.5200	2.117	.999
	Acetates	.5700	-0.4633	.999
$\log K_{o/w} = k_3 T_b + k_4$	Alkanes	0.01748	-1.8210	0.998
	Alkenes	.02110	-3.7309	.999
	Aromatics	.02136	-5.5677	.997
	Acetates	.02306	-7.3981	.999

important in order to correlate other solute properties for estimation purposes such as bioconcentration and soil adsorption.

The advantages in using a  $\log \gamma_\phi^w$  instead of  $\log K_{o/w}$  in defining  $H$  are: (1) solubility data are more readily available in the literature than  $K_{o/w}$  data, and (2)  $\gamma_\phi^w$  is independent of any solvent/water system.

The utility of the gas chromatographic technique for measuring activity coefficients in organic solutions and the validity of the data obtained are now well established. The technique is particularly applicable to measurements at infinite dilution, the condition of primary interest and least accessible otherwise. There are several important advantages to this method for measuring  $\gamma_\phi^o$ : (1) the speed with which the measurements are made, (2) the accuracy of the measurements, (3) the measurements are made at infinite dilution, and (4) several solutes may be injected into the gas chromatograph simultaneously thus increasing the productivity rate. Although absolute  $\gamma_\phi^o$  value may be obtained by this method, the method is best suited for measuring values relative to some carefully studied standards because of the relatively high volatility of octanol.

In the octanol/water system at equilibrium the water phase is saturated with octanol(wo), and the octanol phase is saturated with water(ow). In order to derive an expression relating  $K_{o/w}$  to the solute aqueous solubility,

we assume that  $\gamma_\phi^{wo} \cong \gamma_\phi^o$  and  $\gamma_\phi^{ow} \cong \gamma_\phi^w$  where  $\gamma_\phi^w$  and  $\gamma_\phi^o$  are the solute activity coefficients in pure water and pure octanol, respectively. The extent to which these assumptions are valid may be judged by the very good agreement between  $K_{o/w}$  values calculated via eq (10) and experimental values (table 1) measured by a generator column method [12,13].

The above method for determining  $K_{o/w}$  is best suited for volatile compounds. There are drawbacks to the method for relatively nonvolatile solutes. These compounds depending on their  $\gamma_\phi^o$  value could require a relatively long time to elute through the GC column, thus making the method impractical.

For the cases where vapour pressure data are not available, Purnell has shown that carbon number ( $n_c$ ) or boiling point ( $T_b$ ) may be substituted for  $\log P^\circ$ , i.e.

$$\log K_{o/w} = k_1 n_c + k_2 \quad (18)$$

$$\log K_{o/w} = k_3 T_b + k_4 \quad (19)$$

where the  $k$ 's are constants.

Equation (19) is particularly useful when the homologous series cannot be described by the carbon numbers. The results listed in the table 2 show that there is an excellent correlation with these quantities.

In summary it is clear that the chromatographic technique, particularly in association with the developments reported here, offers a primary route to rapid collection of large volumes of reliable values of  $K_{o/w}$  and other thermodynamic functions associated with the partitioning of organic material in the environment. The infinite dilution method proposed in this paper has all the advantages of a chromatographic method in that there are no stringent demands placed on the purity of the solute or the amount of material required to determine  $K_{o/w}$ .

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